*IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*
*ISSN: 2455-264X, Volume 11, Issue 6 Series II (Nov. – Dec. 2025), PP 11-39*
*www.iosrjournals.org*

# Phytochemical Screening and Molecular Target Binding Ability of some boi-active constituents of *Costus afer*

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***Absract***
*Costus afer is used in ethnomedicine to treat and manage a wide range of diseased conditions. Thirty compounds were identified in the GC/MS analysis of Costus afer stem juice. Among the compound identified are; 2 Furancaboxaldehyde, 5-(hydroxymethyl)- was present in 70.357% forming one of the major constituents in the juice and Dihydro-3-methylene-5-methyl-2-furanone (0.149%) having the least quantity. Gas Chromatography-Mass Spectophotometry analysis of Costus afer aqueous leaves extract revealed the presence of seventy-seven bio-active constituents. Among the compound identified are; 2,4-Di-tert-butylphenol was present in 8.08% forming one of the major constituents in the leaf extract and 3-Eicosene, (E)- (0.15%) having the least quantity. Forty-two bio-active constituents was found in Costus afer aqueous root extract. Among the compound identified are; Heptadecane, 2,6,10,14-tetramethyl was present in 9.40% forming one of the major constituents in the leaf extract and .beta.-Myrcene (0.30%) having the least quantity. Molecular docking revealed the 2,4-Di-tert-butylphenol and Maltol binding affinity on FSH receptor just like urofollitropin which is a standard drug. Based on the critical roles of 2,4-Di-tert-butylphenol and Maltol as anti-inflammatory agents to bring about positive effects on reproduction in inflammation. These phytochemicals; 2,4-Di-tert-butylphenol and Maltol are potential evolving remedies for preventing and treating infertility in male and female respectively. The results show that Costus afer may be an important reservoir for developing affordable fertility drugs.*

***Keywords:*** *Phyto-constituents, Molecular Docking, Costus afer, Anti-inflammatory, Gas Chromatography-mass spectrometry.*

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Date of Submission: 08-12-2025 Date of Acceptance: 20-12-2025

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## I. Introduction

Medicinal plants have long been a cornerstone of traditional medicine, providing a wealth of bioactive compounds that have shaped healing practices across cultures. These natural resources continue to serve as a vital source of therapeutic agents, particularly as interest in plant-based remedies resurges in modern healthcare (Tailulu *et al.,* 2015) [1].

Medicinal and aromatic plants, especially those with ethnopharmacological uses, have been utilized as a natural source of remedies and healthcare for millennia (Noureddine and Lahcen, 2024) [2].

Medicinal plants which are the bedrock of pharmaceutical products have been observed to be cheaper, readily available and exert less adverse side effect compared to orthodox drugs. Rapid progression of resistant microbes and the lack of a strategic management plan have led researchers to consider plant-derived substances (PDS) as alternative or complementing bioactive agent against numerous diseases (Mohamed *et al.,* 2025) [3].

Medicinal plants are employed for their pharmacological or therapeutic properties in ethno-medicine although the scientific basis for the therapeutic benefits are scarce or totally unknown. They harbor several secondary metabolites that are also known as phytochemicals. These plant-derived substances have been reported to be the bioactive compounds that confer the various pharmacological properties of plants on them. . The phytochemicals that are effective in disease prevention and treatment include alkaloids, flavonoids, tannins,

saponins, phytosterols, phenols, polyphenols, cardiac glycosides, terpenoids, anthraquinones, carotenoids and many others (Yuan *et al.,* 2016) [4].

Traditional societies and ethnic nationalities have, over the years, employed medicinal plants in ethnomedicine for the treatment of various diseases without any scientific knowledge of the physiologically active ingredients responsible for the plant's medicinal and pharmacological potentials (Susanti *et al.,* 2023*)* [5]. Science-based use of plants and their extracts to treat or prevent disease, which is also a source of important compounds for drug discovery (Ibrahim et al., 2024) [6] has made outstanding achievements in the attenuation of sickle-cell anaemia, dementia, epilepsy, arthritis, hypertension, Alzheimer's and Parkinson's diseases, malaria, stroke, diabetes and many others **(**Dharmaraj Sharma *et al.,* 2025**)**. The availability of medicinal products from living things, predominantly plants, has interested the pharmacological biochemist as well as the medical biochemist for decades. Phytochemicals or secondary metabolites are chemical compounds that occur naturally in plants and are derived from them. The phytochemical studies of medicinal plants have provided some biochemical basis for their ethnopharmacological uses in treating, managing, and preventing various diseases and disorders [8] (Bhupesh et al., 2021).

*Costus afer* is a rhizomatous herb commonly known as ginger lily or "bush cane" in English, Kaki zuwa by the Hausas, Okpete or Okpoto by the Igbo's and Tete Ogun by the Yorubas, all of Nigeria [9-10] ((Boison *et al.,* 2019; Ijioma *et al*., 2014). Almost every part of this plant is endowed with medicinal potential in diseases such as malaria, measles, diabetes mellitus, arthritis, and stomach disorders. In West Africa especially in for instance, the succulent stem is chewed to quench thirst and also to treat cough and its accompanying sore throat [11] (*Omokhua, 2011)*. Various solvent extracts of the plant leaves, stem, rhizomes, and roots have been studied and reported to contain chemical compounds that could be useful in the alleviation of oxidative stress-related conditions [12-13] (Ukpabi *et al.,* 2012; Anyasor *et al.,* 2014).

Bio-active compounds from medicinal plants are identified, extracted, screened, isolated, and used in production of finished pharmaceutical drugs after proper clinical trials. Molecular docking is one of the scientific techniques that are useful for studying the mechanism of action of important phytochemicals.

Molecular docking is a computational technique that predicts the binding affinity of ligands to receptor proteins. Although it has potential uses in nutraceutical research, it has developed into a formidable tool for drug development. Bioactive substances called nutraceuticals are present in food sources and can be used in the management of diseases. Finding their molecular targets can help in the creation of disease-specific new therapies [14] (Agu *et al.,* 2023). It is a technique that predicts the preferred orientation, affinity, and interaction of a ligand in the binding site of a target protein to form a stable complex (15) (Lengauer and Rarey 1996). It is a bioinformatic modelling strategy critical in drug development, structural biology, and biomolecular interaction research. The associations between biologically relevant molecules such as proteins, peptides, nucleic acids, carbohydrates and lipids play a central role in signal transduction, and the relative orientation of the two interacting partners may affect the type of signal produced, such as agonism or antagonism. Therefore, knowledge of the preferred orientation acquired by docking may be used to predict the strength of association or binding affinity between two molecules and the type of signal produced. Characterization of this binding behaviour plays an important role in the rational design of drugs and in elucidating fundamental biochemical processes [16, 17]. The docking process examines the ligand's spatial and energetic compatibility with the protein receptor's active site, thus assisting in discovering new drug candidates, refining and modifying existing compounds, and understanding the intricate interactions between drugs and receptors. The research thus focuses on computationally simulating the molecular recognition process, which would describe the "best fit" orientation of a ligand that binds to a particular protein of interest. During the docking process, the ligand and the protein adjust their conformation to achieve an overall "best fit," and this conformational adjustment, which results in the overall binding, is referred to as "induced fit" [18].

## II. Materials and Methods

Chemicals and Reagents

All the solvents used were of analytical grade and were procured from Merck, Germany.

**Collection and Authentication of Plant Material**

*Costus*, *afer* plant was harvested from Amuke, Isisala Ngwa South, Abia State. It was authenticated and voucher number obtained (MOUAU/CVM/VPP/HERB/14/008) at department of of forestry, College of Natural Resources and Environmental Management Michael Okpara University of Agriculture Umudike.

**Preparation of *Costus afer* leaves, roots and stem juice Extract**

Leaves were removed from collected stems and washed with distilled water to remove dirt, after which they were debarked. The debarked stems were introduced into a clean manual blender and were crushed to squeeze out their juice. The leaves and root were air dried and ground into a fine powder. Two hundred (200 g) of the powdered leaves and roots were soaked in different 1000 ml of distilled water and allowed to stand for 48 hours with occasional stirring to allow proper extraction.

**GC-MS (Gas Chromatography-Mass Spectrometry) Analysis**

The characterisation of the phytochemicals in *Costus afer* aqueous leaf & root extract and stem juice was done using GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemicals in the sample was carried out using a QP2010 gas chromatography with thermal desorption system, TD 20, coupled with mass spectroscopy (Shimadzu). The ionisation voltage was 70 eV. Gas chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m, XTI-5). The initial column temperature was 80 ºC for 1 min, and then increased linearly at 70 ºC min-1 to 220 ºC, held for 3 min, followed by a linearly increased temperature of 10 ºC min-1 to 290 ºC for 10 min. The temperature of the injection port was 290 ºC and the GC-MS interface was maintained at 290 ºC. The sample was introduced via an all-glass injector working in split mode with a helium carrier gas low rate of 1.2 ml min-1. The identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS [19,20].

## III. Molecular Docking

**Protein Receptor and Ligand Structure Selection**

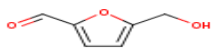
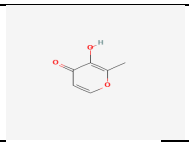
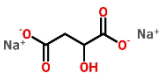
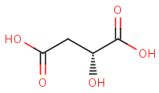
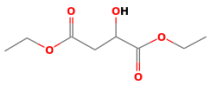
The crystal structure of Follicle stimulating hormone receptor (FSHR) with PDB code 8I2H, was downloaded from RCSB protein data bank and the 3D structure of Ligand compounds; 2,4-Di-tert-butylphenol, Maltol and Urofollitropin with CID code 7311, 8369 and 73759969 respectively were downloaded from PubChem database in sdf format.

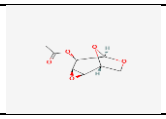
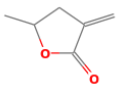
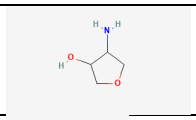
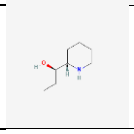
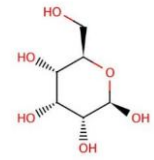
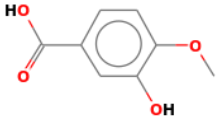
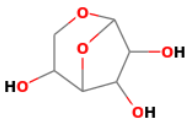
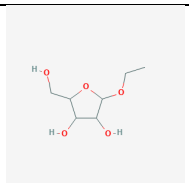
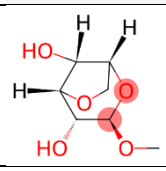
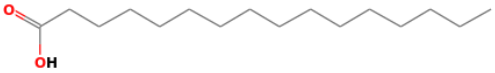
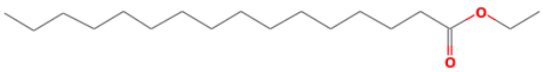
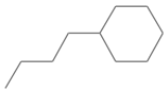
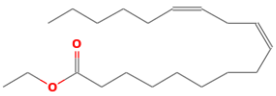
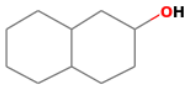
**Procedure:** The crystal structure of FSHR (PDB 8I2H)) was prepared for docking by removing water molecules and co-crystal ligands ssusing Biovia Discovery Studio software. CB-Dock2 software was used for the docking analysis to examine the interactions, binding affinity and binding energy between 2,4-Di-tert-butylphenol (CID 7311) and FSHR as well between Maltol (CID 8369) and FSHR in comparison to interactions between FSHR and Urofollitropin (CID 73759969), a known standard drug. The results of the docking analysis were also perused in 3D and 2D images, using Biovia Discovery Studio software [21].

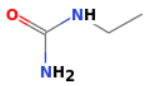
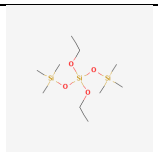
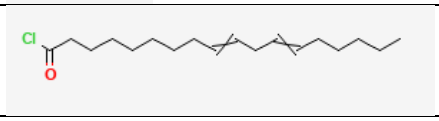
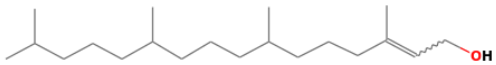
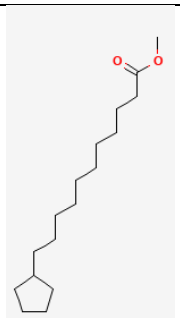
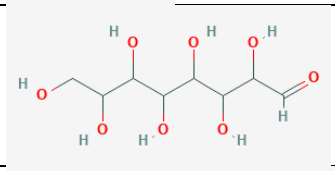
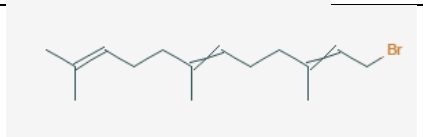
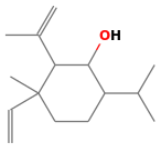
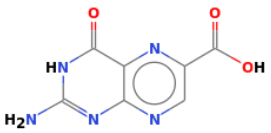
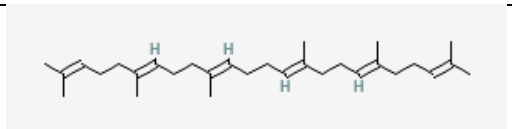
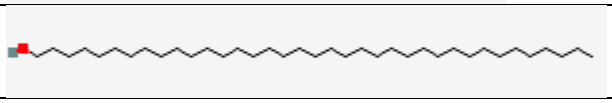
## IV. Results and Discussion

Result above in Table 1 reveals thirty compounds were identified in the GC/MS analysis of *Costus afer* stem juice. The individual names of compounds identified with respect to their individual peak number, retention time, area %, and structure are shown in Table above. Among the compound identified are; 2 Furancaboxaldehyde, 5-(hydroxymethyl)- was present in 70.357% forming one of the major constituents in the juice and Dihydro-3-methylene-5-methyl-2-furanone (0.149%) having the least quantity.

**Table 1:GC-MS Phytochemical analysis of *Costus afer* stem juice**

| PK | RT | Compound | MW | Structure | % Area |
|---|---|---|---|---|---|
| 1 | 5.417 | 2-Furancaboxaldehyde, 5-(hydroxymethyl)- | 126 |  | 70.357 |
| 2 | 5.64 | Maltol | 126 |  | 3.428 |
| 3 | 5.863 | dl-Malic disodiumsalt | 134 |  | 3.041 |
| 4 | 5.904 | Malic acid | 134 |  | 2.288 |
| 5 | 6.002 | Butanedioic acid, hydroxyl-, diethyl ester, (±)- | 190 |  | 0.399 |

| | | | | | |
|---|---|---|---|---|---|
| 6 | 6.024 | 1,6;3,4-Dianhydro-2-O-acetyl-β-d-galactopyranose | 186 |  | 0.788 |
| 7 | 6.369 | Dihydro-3-methylene-5-methyl-2-furanone | 112 |  | 0.149 |
| 8 | 6.466 | Furazan-3-ol, 4-amino- | 101 |  | 0.228 |
| 9 | 6.616 | Conhydrin | 143 |  | 0.515 |
| 10 | 7.381 | D-Allose | 180 |  | 0.163 |
| 11 | 7.711 | 3-Hydroxy-4-methoxybenzoic acid | 168 |  | 0.178 |
| 12 | 7.827 | 1,6-Anhydro-β-D-glucofuranose | 162 |  | 0.153 |
| 13 | 8.266 | Ethyl β-d-riboside | 178 |  | 1.433 |
| 14 | 8.869 | B-D-Glucopyranoside, methyl 3,6-anhydro- | 176 |  | 5.39 |
| 15 | 9.848 | n-Hexadecanoic acid | 256 |  | 0.293 |
| 16 | 10.27 | Hexadecanoic acid, ethyl ester | 284 |  | 4.32 |
| 17 | 10.61 | Cyclohexane, 1-butyl- | 138 |  | 0.22 |
| 18 | 10.76 | Linoleic acid ethyl ester | 308 |  | 0.185 |
| 19 | 11.32 | 2-Naphthalenol, decahydro-, (2α,4aα,8aβ)- | 154 |  | 0.616 |

| | | | | | |
|---|---|---|---|---|---|
| 20 | 11.51 | Urea, ethyl- | 88 |  | 0.222 |
| 21 | 15.01 | Silicic acid, diethyl bis(trimethylsilyl) ester | 296 |  | 0.354 |
| 22 | 15.53 | 9,12-Octadecadienoyl chloride, (Z,Z)- | 298 |  | 1.872 |
| 23 | 17.58 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 296 |  | 0.173 |
| 24 | 18.09 | Cyclopentaneundecanoic acid, methyl ester | 268 |  | 1.553 |
| 25 | 18.19 | I-Gala-I-ido-octose | 240 |  | 0.399 |
| 26 | 18.54 | Farnesyl bromide | 284 |  | 0.19 |
| 27 | 27.1 | 6-epi-shyobunol | 222 |  | 0.258 |
| 28 | 27.12 | Pterin-6-carboxylic acid | 207 |  | 0.375 |
| 29 | 31.95 | Squalene | 410 |  | 0.184 |
| 30 | 34.35 | 1-Heptatriacotanol | 536 |  | 0.276 |

Result above in Table 1 reveals thirty compounds were identified in the GC/MS analysis of *Costus afer* stem juice. The individual names of compounds identified with respect to their individual peak number, retention time, area %, and structure are shown in Table above. Among the compound identified are; 2 Furancaboxaldehyde, 5-(hydroxymethyl)- was present in 70.357% forming one of the major constituents in the juice and Dihydro-3-methylene-5-methyl-2-furanone (0.149%) having the least quantity.

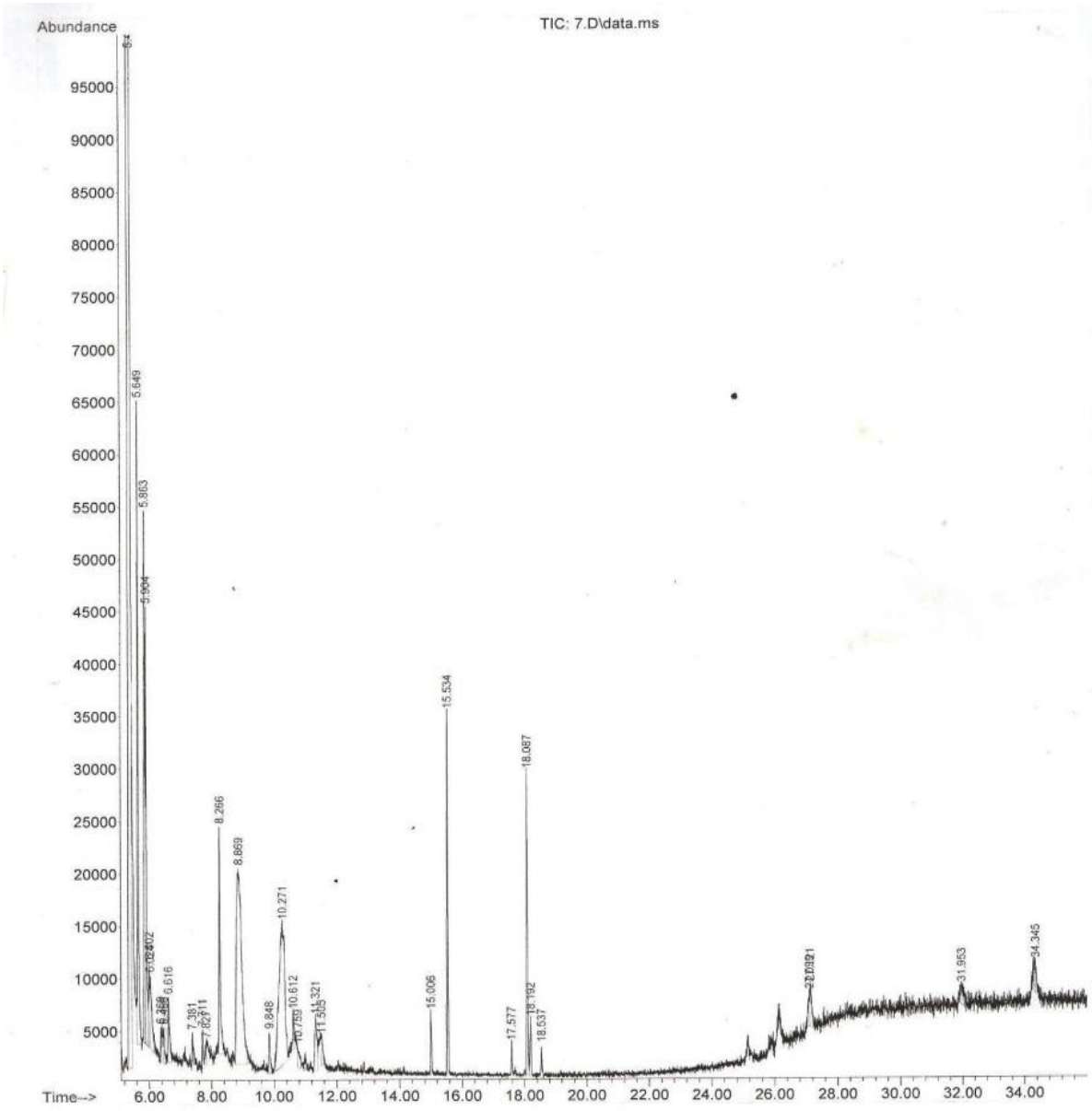
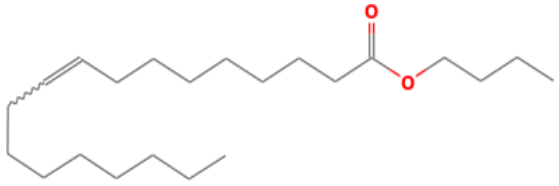
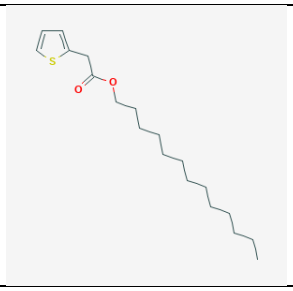
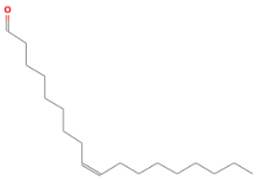
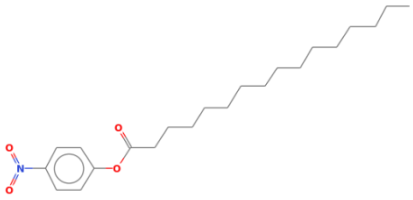
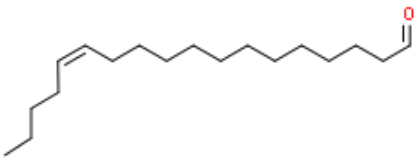
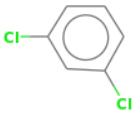
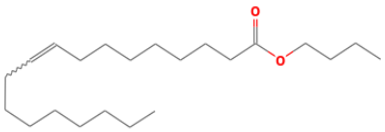
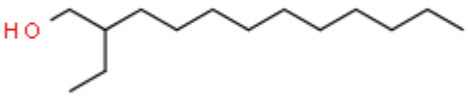
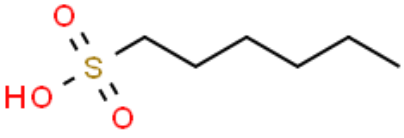
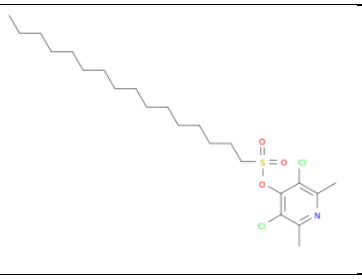
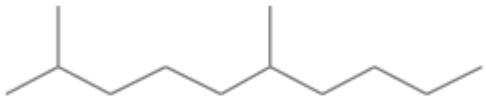
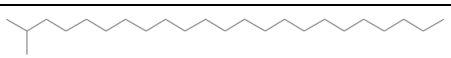
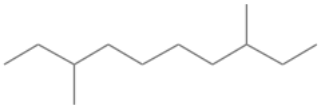
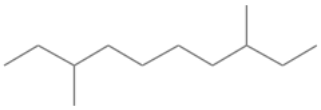
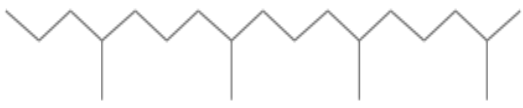
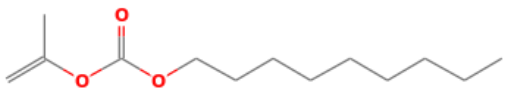
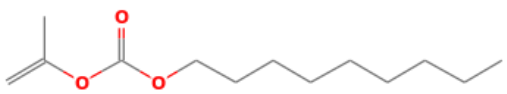
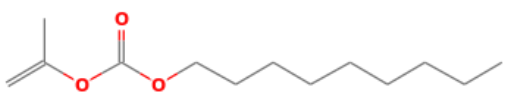


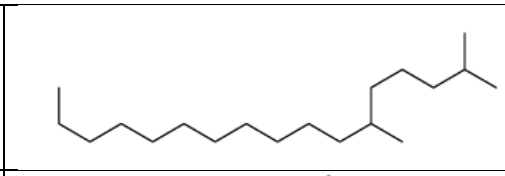
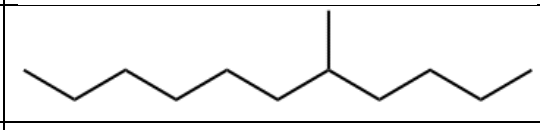
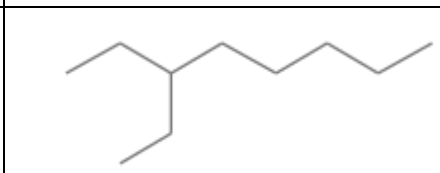
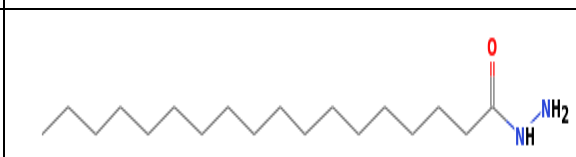
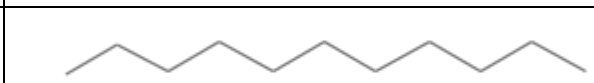
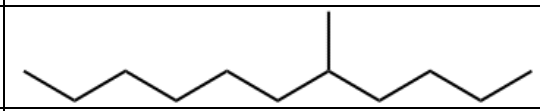
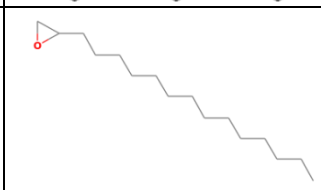
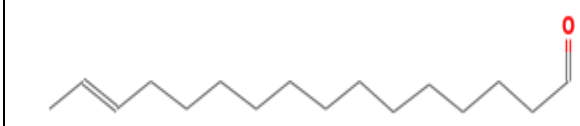
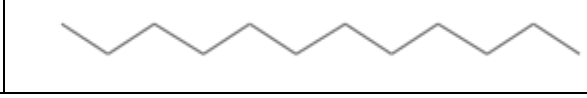

**Figure 1. GC-MS chromatogram of crude stem juice extract of *Costus afer.***

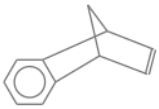
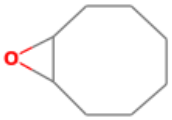
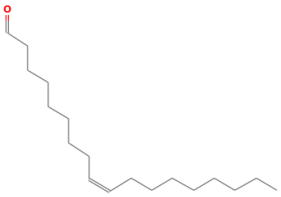
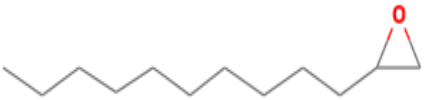
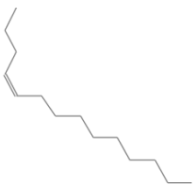
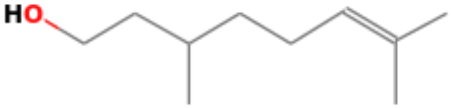
**Table 2: GC-MS Phytochemical analysis of aqueous *Costus afer* leaf extract**

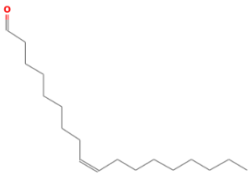
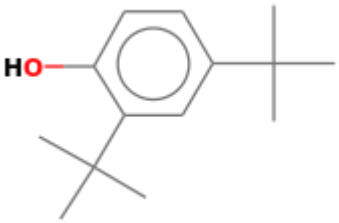
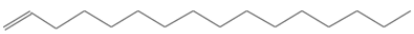
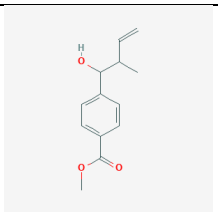
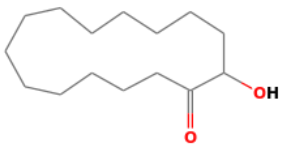
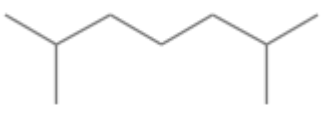
| PK | RT | Compound name | Molecular weight | Structure | Area (%) |
|---|---|---|---|---|---|
| 1 | 5.250 | Butyl 9-octadecenoate | 338.6 |  | 1.49 |

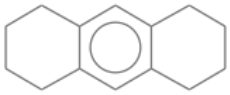
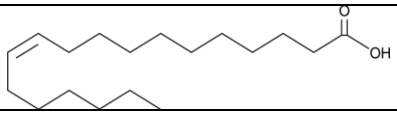
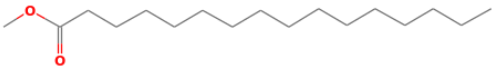
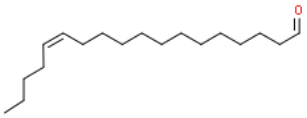
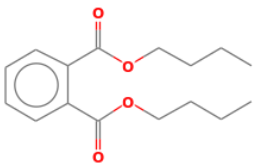
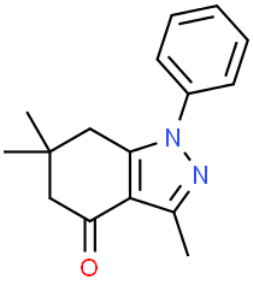
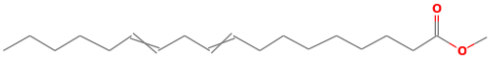
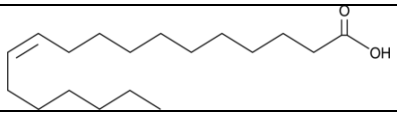
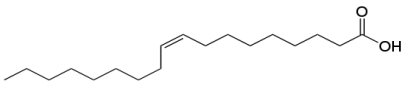
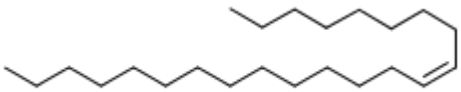
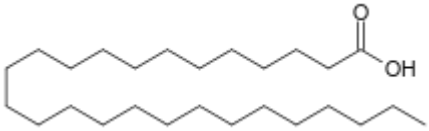
| | | | | | |
|---|---|---|---|---|---|
| 2 | 5.455 | 2-Thiopheneacetic acid, 3-tridecyl ester | 324.5 |  | 0.17 |
| 3 | 6.885 | 9-Octadecenal | 266.4620 |  | 1.05 |
| 4 | 7.137 | Hexadecanoic acid, 4-nitrophenyl ester | 377.5176 |  | 0.12 |
| 5 | 7.225 | 13-Octadecenal | 266.462. |  | 0.12 |
| 6 | 7.425 | Benzene 1,3- dichloro- | 147.002 |  | 1.33 |
| 7 | 7.788 | Octadecane, 1-(ethenyloxy)- | 296.5310 |  | 0.59 |
| 8 | 8.362 | Butyl 9-octadecenoate | 338.6 |  | 0.64 |
| 9 | 8.521 | 2-Ethyl-1-dodecanol | 214.3874 |  | 1.41 |
| 10 | 8.612 | 1-Hexadecanesulfonic acid | 328.48 |  | 2.07 |

| | | | | | |
|---|---|---|---|---|---|
| 11 | 8.876 | Hexadecane, 3-methyl-3,5--dichloro-2-6-dimethyl-4-pyridyl ester | 480.53166 |  | 3.36 |
| 12 | 8.980 | Nonane | 128.2551 |  | 1.84 |
| 13 | 9.059 | Decane | 142.29 |  | 1.19 |
| 14 | 9.138 | 2,6-Dimethyldecane | 170.3348 |  | 1.69 |
| 15 | 9.217 | Tricosane, 2-methyl- | 338.6538 |  | 0.96 |
| 16 | 9.430 | Decane, 3,8-dimethyl | 170.3348 |  | 7.72 |
| 17 | 9.558 | Decane, 3,8-dimethyl | 170.3348 |  | 1.66 |
| 18 | 9.622 | Undecane | 156.31 |  | 2.93 |
| 19 | 9.737 | Dodecane | 170.34 |  | 3.16 |
| 20 | 9.816 | Heptadecane, 2,6,10,14-tetramethyl | 296.5741 |  | 4.71 |
| 21 | 9.938 | Carbonic acid, nonyl prop-1-en-2-yl ester | 228.3279 |  | 1.24 |
| 22 | 9.938 | Carbonic acid nonyl prop-1-en-2-yl ester | 228.3279 |  | 1.24 |
| 23 | 10.159 | Carbonic acidnonyl prop-1-en-2-yl ester | 228.3279 |  | 3.73 |

| | | | | | |
|---|---|---|---|---|---|
| 24 | 10.268 | Heptadecane, 2,6-dimethyl- | 268.521 |  | 4.51 |
| 25 | 10.506 | Undecane, 5- methyl- | 170.33 |  | 1.70 |
| 26 | 10.579 | Tricosane, 2-methyl- | 338.7 |  | 1.30 |
| 27 | 10.635 | Octane, 3-ethyl- | 142.2817 |  | 1.52 |
| 28 | 10.992 | Stearic acid hydrazide | 298.5071 |  | 0.26 |
| 29 | 11.119 | Undecane | 156.31 |  | 0.26 |
| 30 | 11.336 | Undecane, 5-methyl- | 170.33 |  | 0.35 |
| 31 | 11.468 | Oxirane, tetradecyl- | 240.4247 |  | 0.15 |
| 32 | 11.563 | 1-Octadecanesulphonyl chlorid | 353.003 |  | 0.19 |
| 33 | 11.710 | Stearic acid hydrazide | 298.5071 |  | 0.07 |
| 34 | 12.584 | E-14-Hexadecenal | 238.4088 |  | 0.95 |
| 35 | 12.803 | Dodecane | 170.3348 |  | 1.73 |

| | | | | | |
|---|---|---|---|---|---|
| 36 | 13.191 | Trifluoroacetoxy hexadecane | 338.44863 |  | 0.24 |
| 37 | 14.651 | Stearic acid hydrazide | 298.5071 |  | 0.33 |
| 38 | 14.888 | Decyloctyl ether | 270.4937 |  | 0.57 |
| 39 | 15.706 | Tridecane | 184.3614 |  | 1.82 |
| 40 | 15.873 | 1,4-Methanonaphthalene, 1,4-dihydro- | 142.1971 |  | 0.84 |
| 41 | 16.361 | 9-Oxabicyclo[6.1.0]nonane | 126.1962 |  | 0.53 |
| 42 | 17.108 | 9-Octadecenal, (Z)- | 266.4620 |  | 0.17 |
| 43 | 17.473 | Hexadecane, 1-chloro- | 260.886 |  | 0.26 |
| 44 | 17.804 | Oxirane, decyl- | 184.3184 |  | 0.23 |
| 45 | 18.283 | 4-Tetradecene, (Z)- | 196.3721 |  | 1.31 |
| 46 | 18.476 | Tridecane | 184.3614 |  | 1.33 |
| 47 | 19.354 | Citronellol | 156.27 |  | 0.23 |

| | | | | | |
|---|---|---|---|---|---|
| 48 | 19.437 | Naphthalene, 2,7-dimethyl- | 156.2237 |  | 0.34 |
| 49 | 19.743 | 1-Hexacosanol | 382.7064 |  | 0.15 |
| 50 | 20.087 | 9-Octadecenal | 266.4620 |  | 0.46 |
| 51 | 20.899 | Dodecane, 1,1'-oxybis- | 354.6532 |  | 0.16 |
| 52 | 21.104 | Undecane, 2-methyl- | 170.3348 |  | 0.88 |
| 53 | 22.130 | 2,4-Di-tert-butylphenol | 206.3239 |  | 8.08 |
| 54 | 23.440 | Z-8-Hexadecene | 224.4253 |  | 2.35 |
| 55 | 23.743 | Cetene | 224.4253 |  | 0.31 |
| 56 | 23.978 | Cyclohexadecane | 224.4253 |  | 0.30 |
| 57 | 25.513 | Benzoic acid, 4-(3-hydoxy-3-methyl-1-butynyl)-, methyl ester | 220.26 |  | 0.32 |
| 58 | 25.958 | Cyclopentadecanone, 2-hydroxy- | 240.3816 |  | 0.26 |
| 59 | 26.072 | Heptane, 2,6-dimethyl- | 128.255 |  | 0.41 |

| | | | | | |
|---|---|---|---|---|---|
| 60 | 28.090 | 1-Octadecene | 252.4784 |  | 2.66 |
| 61 | 28.553 | 3-Eicosene, (E)- | 280.5316 |  | 0.15 |
| 62 | 29.799 | Anthracene, 1,2,3,4,5,6,7,8-octahydro-1,1,4,4,5,5,8,8-0ctamethyl- | 186.2927 |  | 0.47 |
| 63 | 29.928 | cis-Vaccenic acid | 282.46. |  | 0.20 |
| 64 | 30.069 | Hexadecanoic acid, methyl ester | 270.4507 |  | 1.02 |
| 65 | 30.415 | 13-Octadecenal, (Z)- | 266.462. |  | 0.16 |
| 66 | 30.527 | Dibutyl phthalate | 278.34 |  | 1.31 |
| 67 | 30.886 | Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro- | 306.4 |  | 0.60 |
| 68 | 31.551 | 9,12-Octadecadienoic acid, methyl ester | 294.4721 |  | 1.43 |
| 69 | 31.762 | cis-Vaccenic acid | 282.46. |  | 0.30 |
| 70 | 32.181 | 1-Docosene | 308.5848 |  | 1.03 |
| 71 | 32.843 | Oleic Acid | 282.46 |  | 0.61 |
| 72 | 33.375 | 9-Tricosene, (Z)- | 322.6113 |  | 1.08 |
| 73 | 33.550 | Hexacosanoic acid | 396.69 |  | 0.20 |

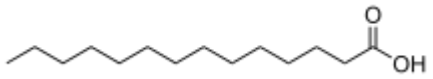
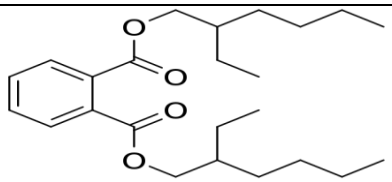
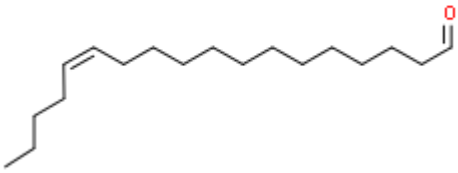
| | | | | | |
|---|---|---|---|---|---|
| 74 | 34.116 | Tetradecanoic acid, dodecyl ester | 396.6899 |  | 0.36 |
| 75 | 34.543 | Bis(2-ethylhexyl) phthalate | 390.5561 |  | 1.70 |
| 76 | 34.796 | 1-Hexacosene | 364.6911 |  | 0.91 |
| 77 | 37.248 | 13- Octadecenal, (z)- | 266.462. |  | 0.33 |

Table 2: GC-MS Phytochemical analysis of ***Costus afer*** **leaves** shows the presence of seventy-seven bio-active constituents. The individual names of compounds identified with respect to their individual peak number, retention time, area %, and structure are shown in table above. Among the compound identified are; 2,4-Di-tert-butylphenol was present in 8.08% forming one of the major constituents in the leaf extract and 3-Eicosene, (E)- (0.15%) having the least quantity

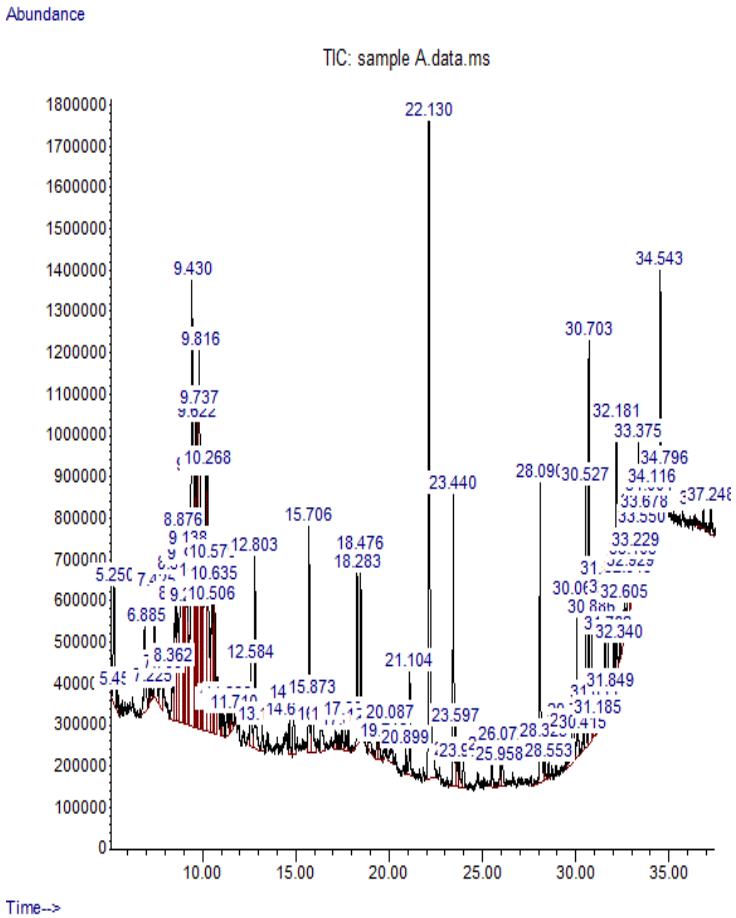
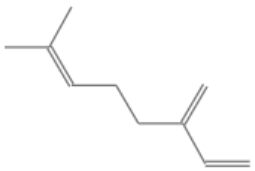
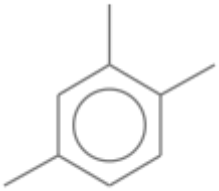
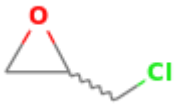
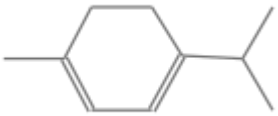
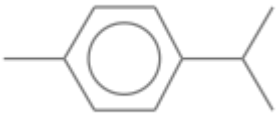
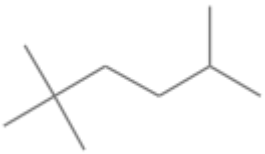
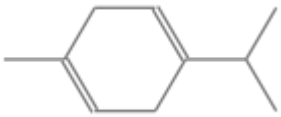
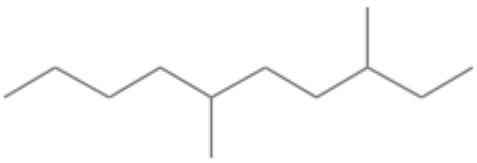
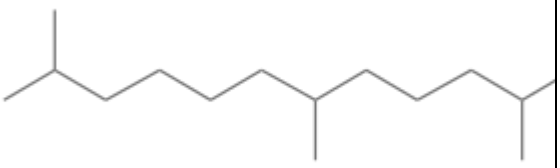
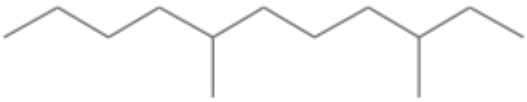
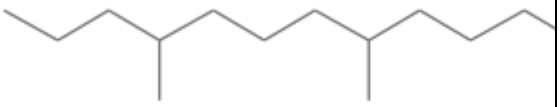
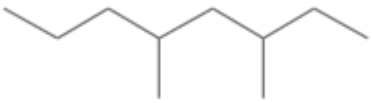
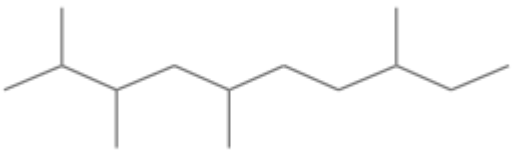
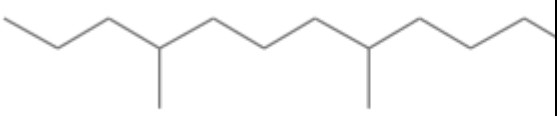
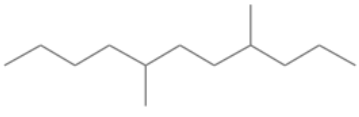
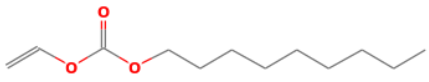
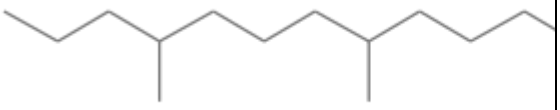
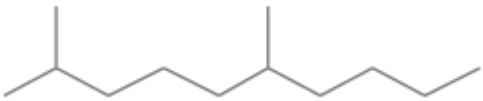
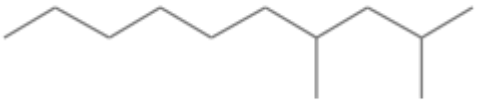
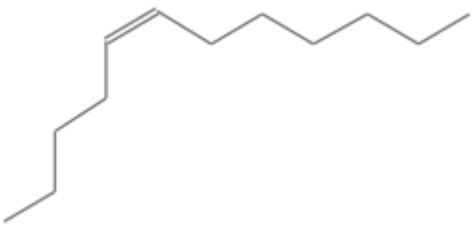
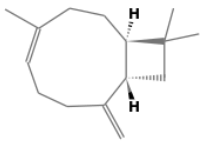
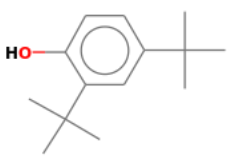


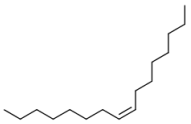
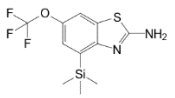
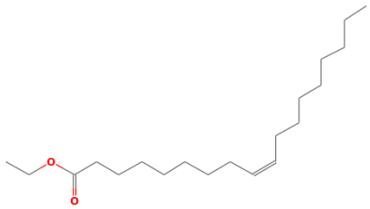
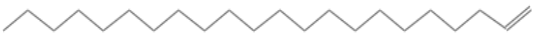
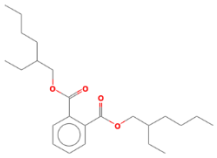
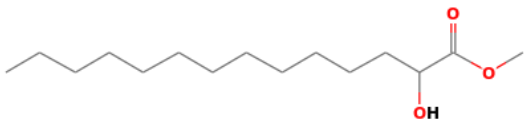
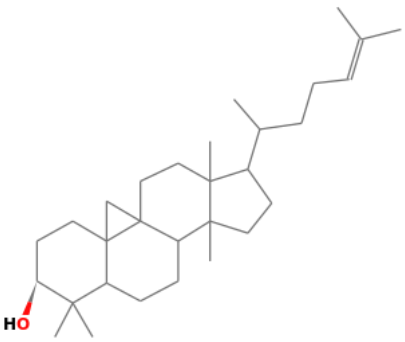

**Fig : 2 chromatogram of GC-MS analysis of *Costus afer* leaves:** shows the retention time, % area and various peaks of the bioactive compounds.

**Table 3: GC-MS Phytochemical analysis of *Costus afer* aqueous root extract.**

| Pk | RT | Compound name | Molecular weight | Structure | Area (%) |
|---|---|---|---|---|---|
| 1 | 6.301 | .beta.-Myrcene | 136.2340 |  | 0.30 |
| 2 | 6.365 | Benzene, 1,2,4-trimethyl- | 120.1916 |  | 0.60 |
| 3 | 6.496 | Oxirane, (chloromethyl)- | 92.524 |   | 1.04 |
| 4 | 6.849 | Benzene, 1,4-dichloro- | 147.002 |   | 1.56 |
| 5 | 6.956 | 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- | 136.2340 |  | 0.73 |
| 6 | 7.201 | p-Cymene | 134.2182 |  | 1.32 |
| 7 | 7.949 | Hexane, 2,2,5-trimethyl- | 128.2551 |  | 0.42 |
| 8 | 8.160 | .gamma.-Terpinene | 136.2340 |  | 4.07 |

| | | | | | |
|---|---|---|---|---|---|
| 9 | 8.258 | Decane, 3,6-dimethyl- | 170.3348 |  | 1.19 |
| 10 | 8.382 | Dodecane, 2,6,11-trimethyl- | 212.4146 |  | 3.58 |
| 11 | 8.536 | Decane | 142.2817 |  | 3.22 |
| 12 | 8.645 | Tridecane | 184.3614 |  | 1.09 |
| 13 | 8.700 | Undecane, 3,7-dimethyl- | 184.3614 |  | 1.55 |
| 14 | 8.958 | Heptadecane, 2,6,10,14-tetramethyl | 296.5741 |  | 9.40 |
| 15 | 9.120 | Octane, 3,5-dimethyl- | 142.2817 |  | 2.11 |
| 16 | 9.178 | Decane, 2,3,5,8-tetramethyl- | 198.3880 |  | 3.39 |
| 17 | 9.273 | Heptadecane, 2,6,10,14-tetramethyl | 296.5741 |  | 3.43 |
| 18 | 9.339 | Tetradecane | 198.3880 |  | 5.13 |
| 19 | 9.486 | Undecane, 4,7-dimethyl- | 184.3614 |  | 1.35 |

| | | | | | |
|---|---|---|---|---|---|
| 20 | 9.543 | Carbonic acid, nonyl vinyl ester | 214.3013 |  | 1.30 |
| 21 | 9.806 | Undecane | 156.3083 |  | 8.02 |
| 22 | 9.917 | Heptadecane, 2,6,10,14-tetramethyl | 296.5741 |  | 0.59 |
| 23 | 10.026 | 2,6-Dimethyldecane | 170.3348 |  | 1.52 |
| 24 | 10.101 | Decane, 2,4-dimethyl- | 170.3348 |  | 2.64 |
| 25 | 12.027 | 5-Dodecene, (Z)- | 168.3190 |  | 0.51 |
| 26 | 12.262 | Dodecane | 170.3348 |  | 1.80 |
| 27 | 15.109 | Tridecane | 184.3614 |  | 1.89 |
| 28 | 17.625 | Cetene | 224.4253 |  | 1.65 |
| 29 | 18.346 | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4 Z,9S*)]- | 204.3511 |  | 0.65 |
| 30 | 20.413 | Pentadecane | 212.4146 |  | 1.01 |
| 31 | 20.974 | 2,4-Di-tert-butylphenol | 206.3239 |  | 9.27 |

| | | | | | |
|---|---|---|---|---|---|
| 32 | 22.690 | Z-8-Hexadecene | 224.425 |  | 3.16 |
| 33 | 22.860 | Hexadecane | 226.4412 |  | 0.50 |
| 34 | 27.258 | 1-Octadecene | 252.4784 |  | 3.94 |
| 35 | 30.022 | 1,2-Benzenedicarboxylic acid, l 2-ethylhexyl ester | | | 1.18 |
| 36 | 30.433 | 6-(Trifluoromethoxy)-N-(trimethylsilyl)-1,3-benzothiazol-2-amine | 306.38 |  | 0.62 |
| 37 | 31.666 | Ethyl Oleate | 310.5145 |  s | 0.50 |
| 38 | 31.814 | 1-Docosene | 308.5848 |  | 1.54 |
| 39 | 34.008 | Bis(2-ethylhexyl) phthalate | 390.5561 |  | 1.50 |
| 40 | 34.216 | Tetradecanoic acid, 2-hydroxy-, methyl ester | 258.3969 |  | 1.11 |
| 41 | 35.582 | 9,19-Cyclolanost-24-en-3-ol, (3.beta.)- | 426.7174 |  | 2.11 |

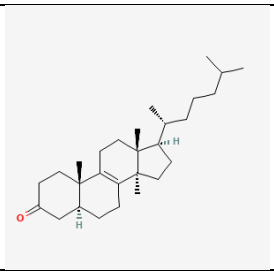
| | | | | | |
|---|---|---|---|---|---|
| 42 | 36.021 | 5.alpha.-Cholest-8-en-3-one, 14-methyl- | |  | 2.23 |

Table 3 shows GC-MS Phytochemical analysis of *Costus afer* roots which reveals the presence of forty-two bio-active constituents. The individual names of compounds identified with respect to their individual peak number, retention time, area %, and structure are shown in table above. Among the compound identified are; Heptadecane, 2,6,10,14-tetramethyl was present in 9.40% forming one of the major constituents in the leaf extractand .beta.-Myrcene (0.30%) having the least quantity

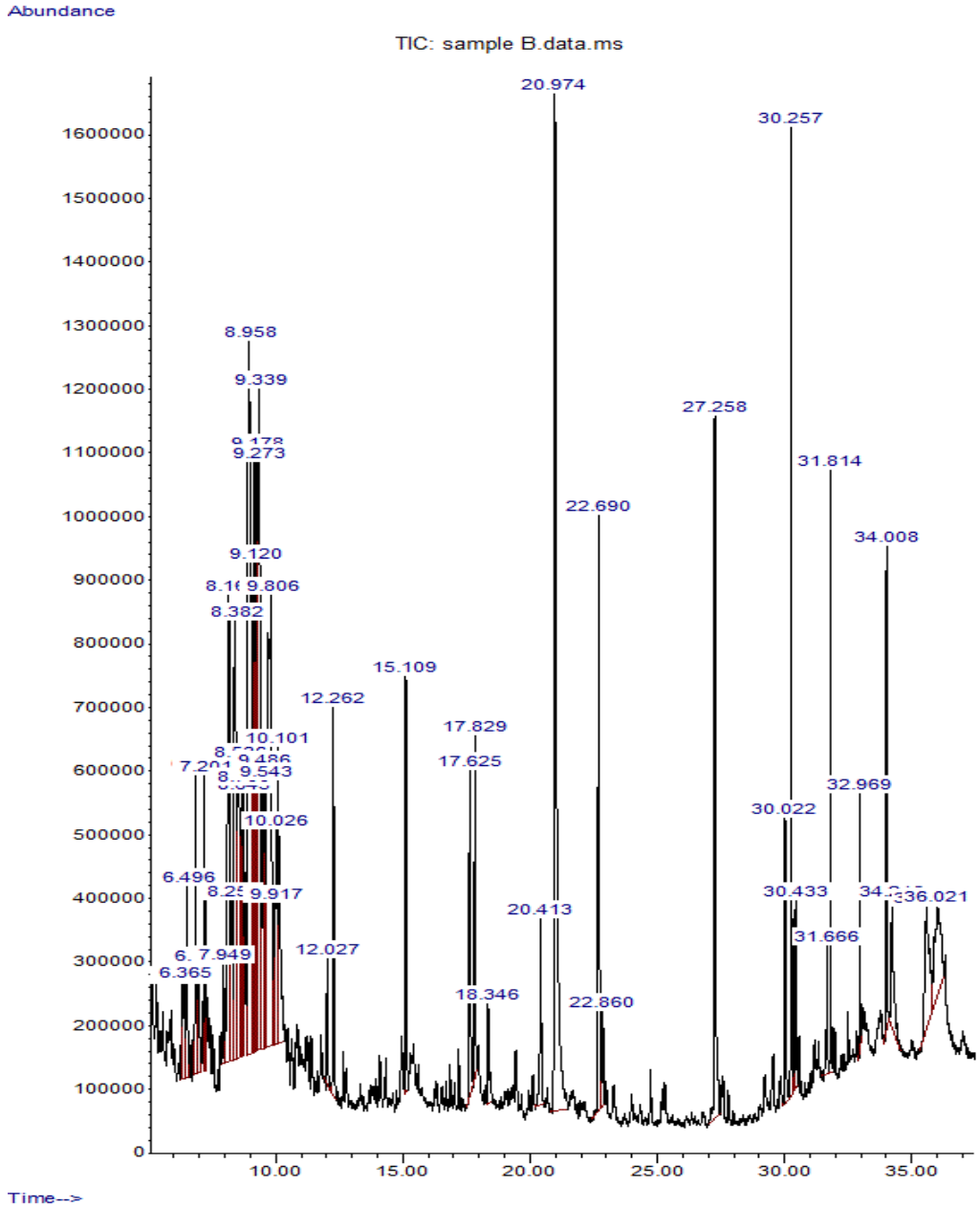



**Fig : 3 chromatogram of GC-MS analysis of *Costus afer* root:** shows the retention time, % area and various peaks of the bioactive compounds.

**Molecular Docking Analysis**

**Table: 4: Represents the binding complex between FSH receptor and ligand compounds 2,4-Di-tert-butylphenol (CID: 7311), Maltol (CID: 8369) and Urofollitropin (CID:73759969) showing vina score and hydrogen bonds at different positions.**

| | 2,4-Di-tert-butylphenol CID:7311 | | Maltol CID: 8369 | | Urofollitropin CID:73759969 | |
|---|---|---|---|---|---|---|
| POSITIONS | VINASCORE | H BOND | VINASCORE | H BOND | VINASCORE | H BOND |
| C1 | -5.3 | 0 | -4.1 | 2 | -1.9 | 6 |
| C2 | -5.8 | 1 | -4.0 | 0 | -8.1 | 4 |
| C3 | -5.9 | 1 | -4.5 | 2 | -6.9 | 0 |
| C4 | -5.0 | 0 | -3.8 | 2 | -9.0 | 6 |
| C5 | -4.5 | 1 | -4.0 | 1 | -8.8 | 10 |

Binding complex between FSH receptor and ligand compounds 2,4-Di-tert-butylphenol (CID: 7311) and (Maltol CID: 8369) showing vina score and hydrogen bonds at different positions.

The docking results of PDB 8I2H and CID 7311 showed the highest binding affinity at position C3 with lowest scoring function value of -5.9, while CID 8369 at position C3 showed scoring function value of -4.5. However, the interactions between CID 73759969 and PDB 8I2H showed highest binding affinity at position 4 with lowest scoring function value of -9.0.

The high binding affinity between PDB 8I2H and CID 73759969 suggests that low binding energy is required for such interactions, especially at the binding sites represented as positions C4, C5, C2 and C3. However, the scoring function value of -5.9 observed at position C3 between PDB 8I2H and CID 7311 falls within the range of a strong interaction when compared to scoring function value of -6.9 observed at position C3 between PDB 8I2H and CID 73759969. The interaction of these ligand compounds with FSHR may act as a trigger that affects how FSH hormone is secreted in the body.
Protein Receptor: Follicle stimulating hormone receptor (FSHR)
PDB Code: 8I2H

Ligand : 2,4-Di-tert-butylphenol (Present in leaves and root)
Compound CID: 7311

Ligand: Maltol
Compound CID: 8369 (Present in stem)

Ligand: Urofollitropin
Compound CID: 73759969 (Standard drug)

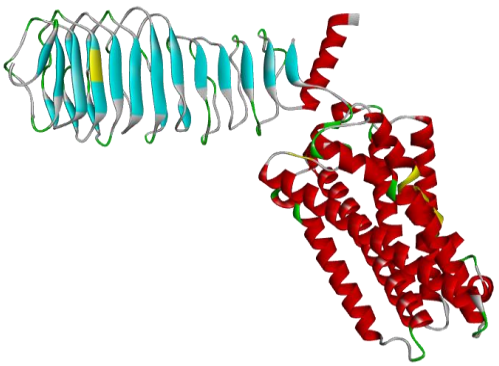


FIGURE 4: 3D DIAGRAM OF CRYSTAL STRUCTURE OF FSHR (PDB: 8I2H)

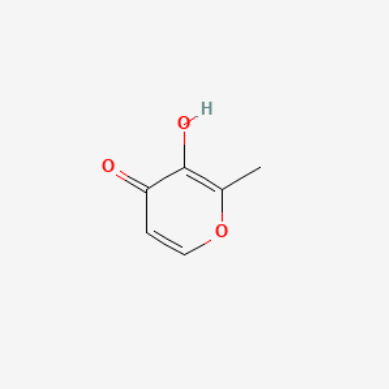


FIGURE 5.1: 2D DIAGRAM OF CID 8369

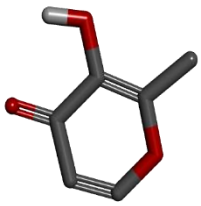


FIGURE 5.2: 3D DIAGRAM OF CID 8369

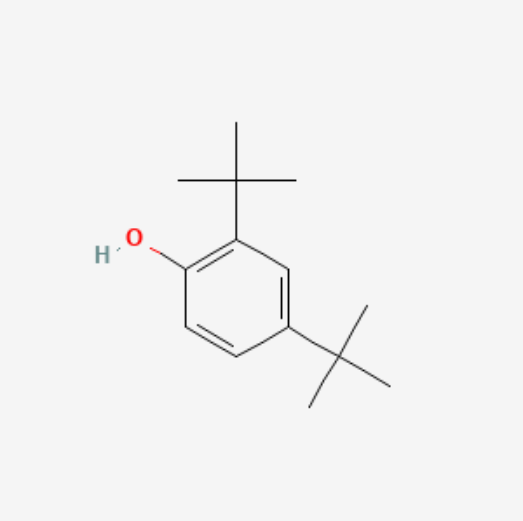


FIGURE 6: 1D DIAGRAM OF CID 7311

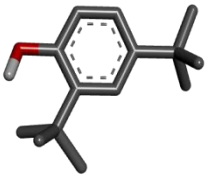


FIGURE 6.2: 3D DIAGRAM OF CID 7311

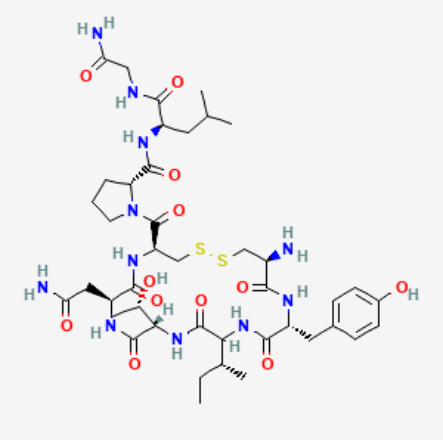


FIGURE 7.1: 2D DIAGRAM OF CID 73759969

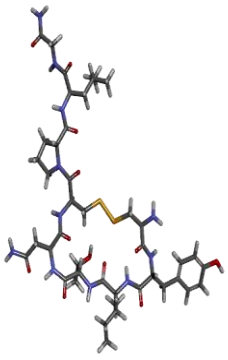


FIGURE 7.2: 3D DIAGRAM OF CID 73759969

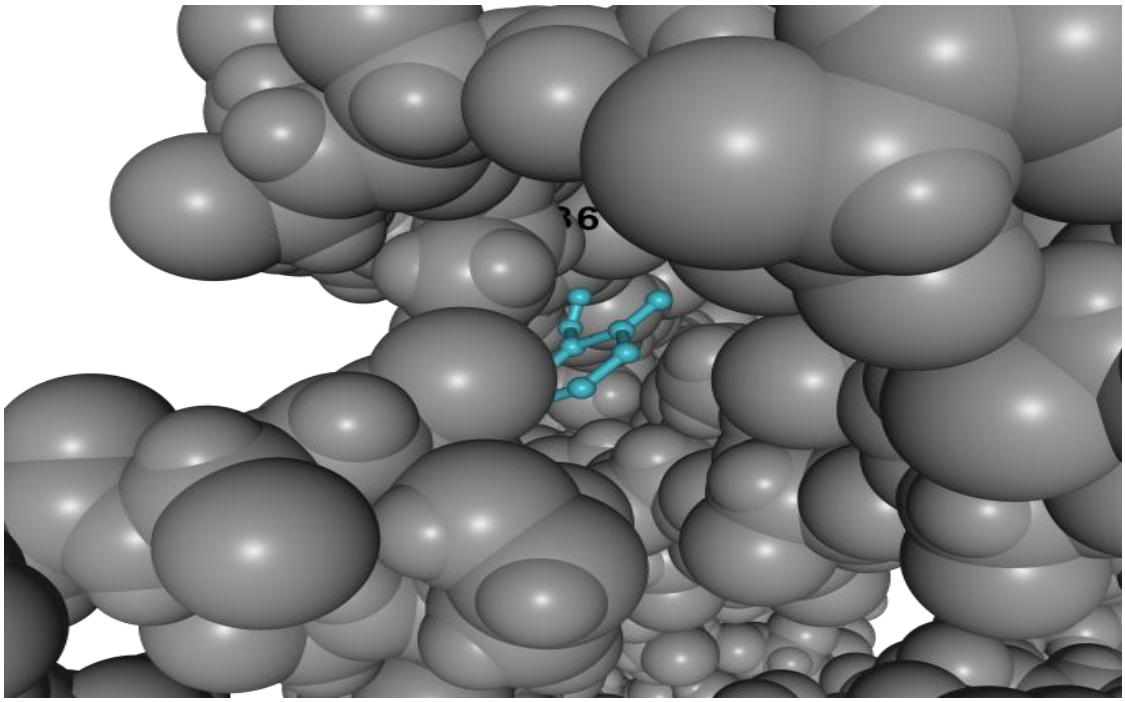


FIG 8.1: 3D IMAGE OF PDB 8I2H AND CID 8369 COMPLEX

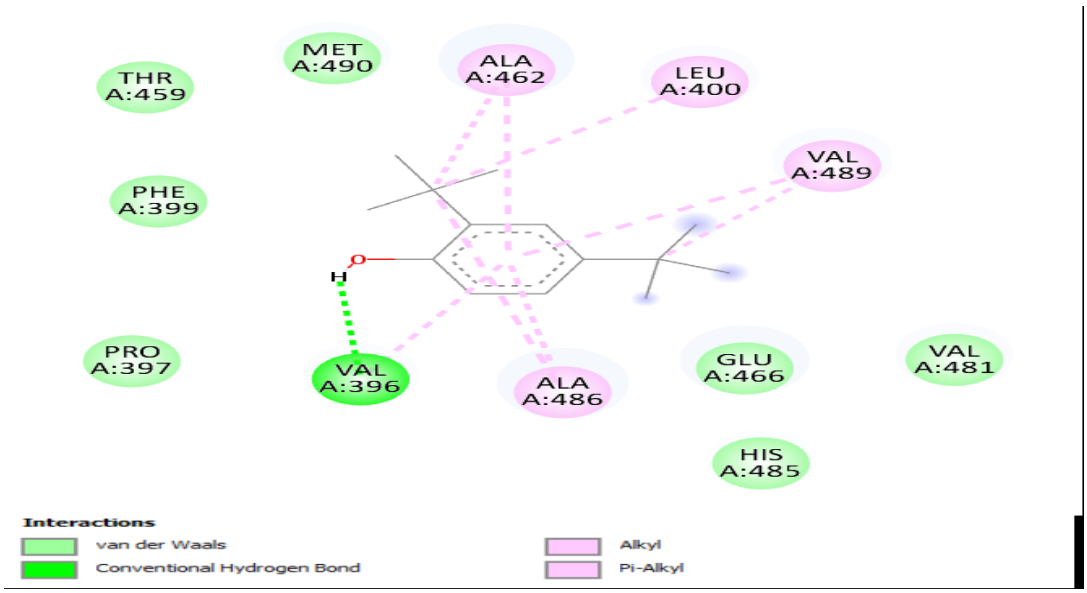



FIG. 8: 2D IMAGE OF PDB 8I2H AND CID 7311 COMPLEX

Fig 8.1 and 8.2 above represents the binding complex between FSHR and 2,4-Di-tert-butylphenol at position C3 with vina score of -5.9 and therefore has the highest binding affinity.

Below are 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (2,4-Di-tert-butylphenol CID:7311) at positions C1-C5 showing binding affinity and bonds at each binding site.

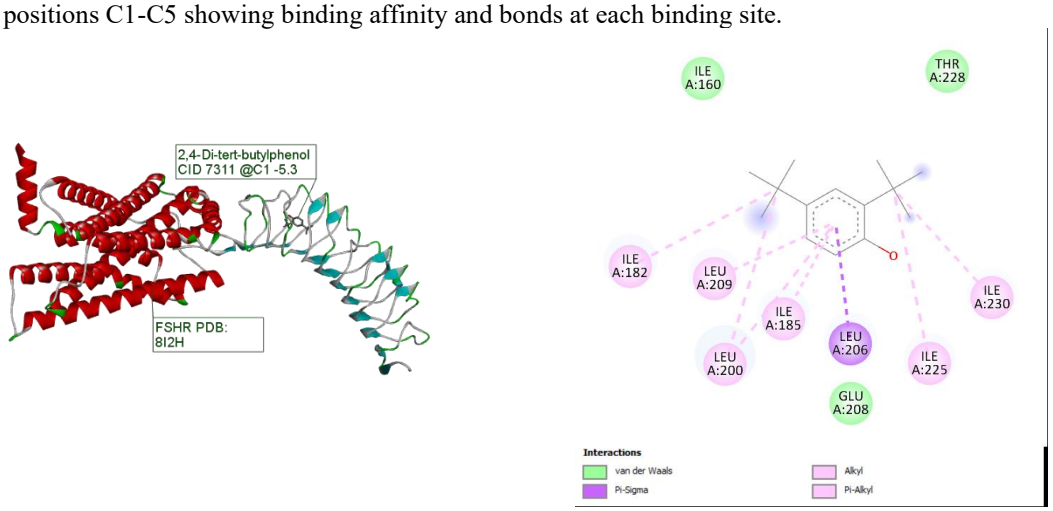



Fig 9.1: 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (2,4-Di-tert-butylphenol CID:7311) at positions C1 showing binding affinity and bonds at the binding site.

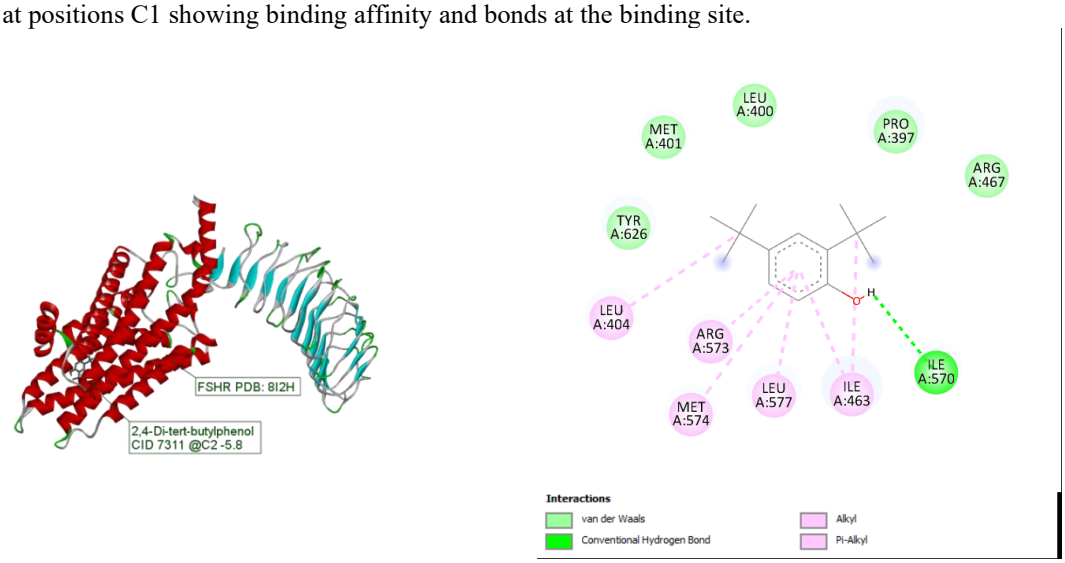



Fig 9.2: 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (2,4-Di-tert-butylphenol CID:7311) at positions C2 showing binding affinity and bonds at the binding site

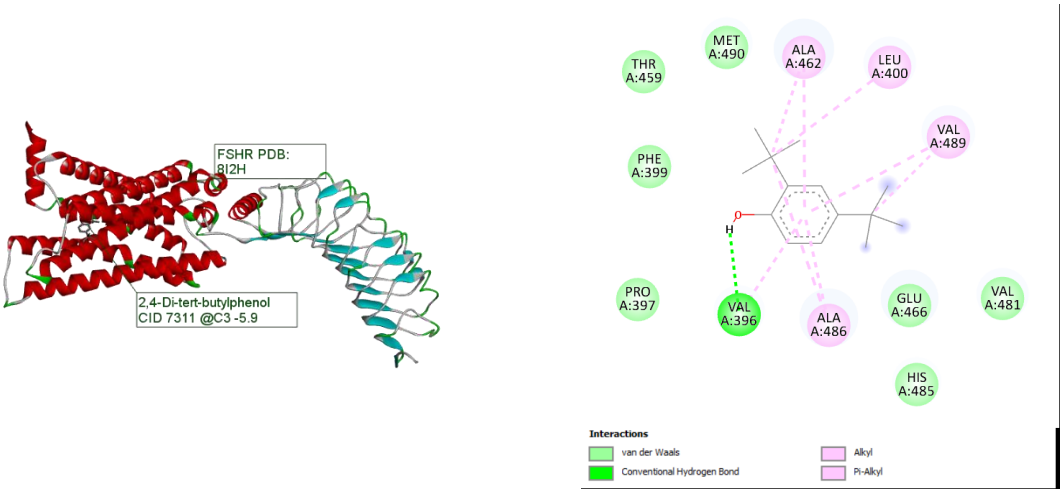



Fig 9.3: 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (2,4-Di-tert-butylphenol CID:7311) at positions C3 showing binding affinity and bonds at each binding site respectively.

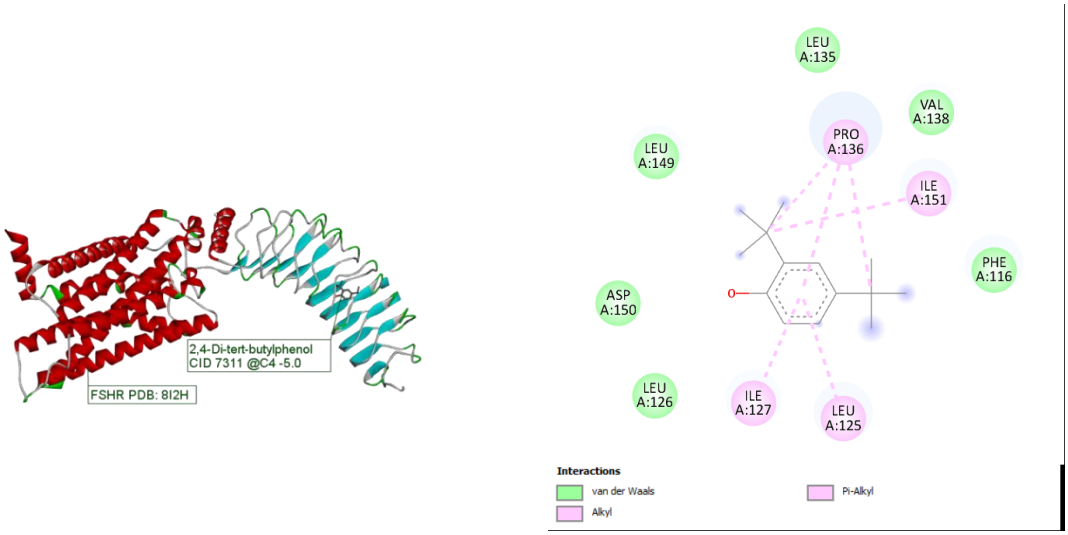



Fig 9.4: 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (2,4-Di-tert-butylphenol CID:7311) at positions C4 showing binding affinity and bonds at each binding site respectively.

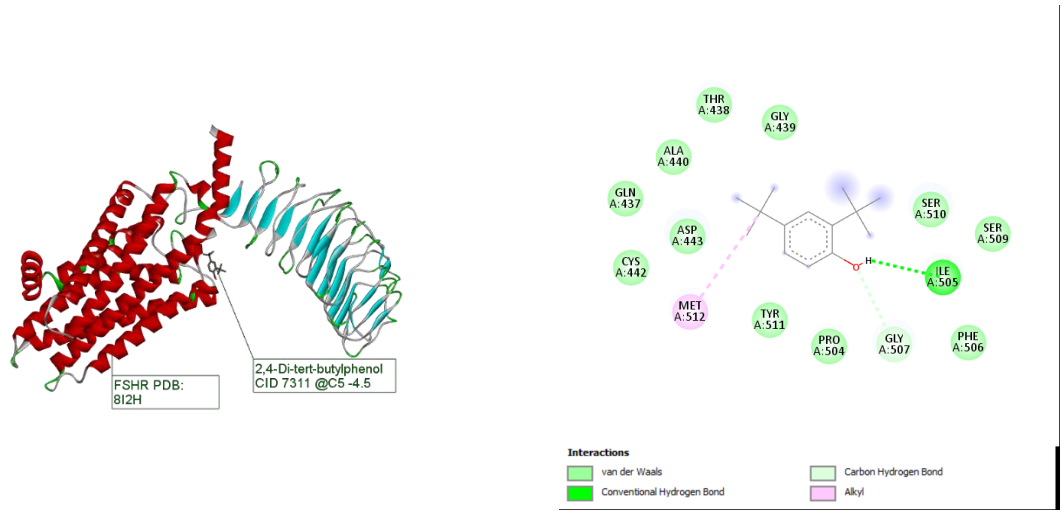



Fig 9.5: 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (2,4-Di-tert-butylphenol CID:7311) at positions C5 showing binding affinity and bonds at each binding site respectively.

Below are 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (Maltol CID: 8369) at positions C1-C5 showing binding affinity and bonds at each binding site.

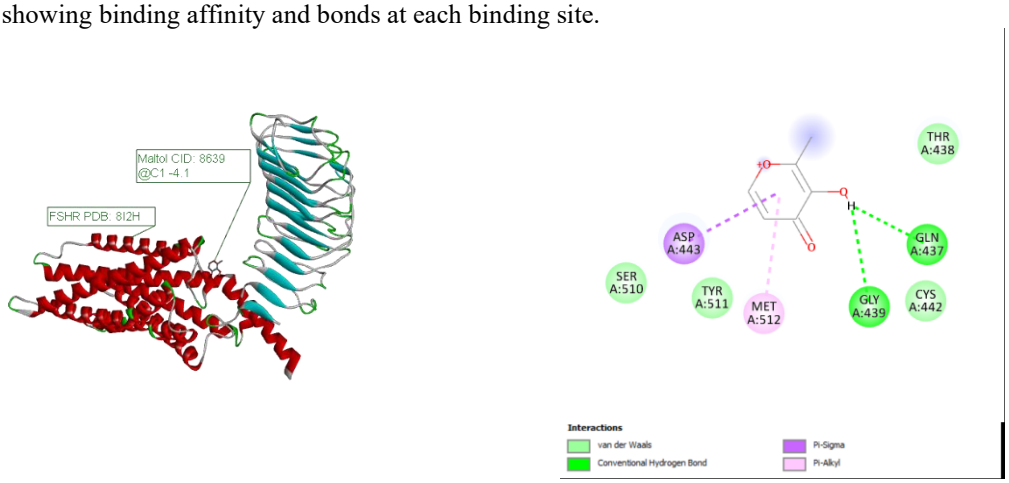



Fig 10.1: 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (Maltol CID: 8369) at positions C1 showing binding affinity and bonds at the binding site.

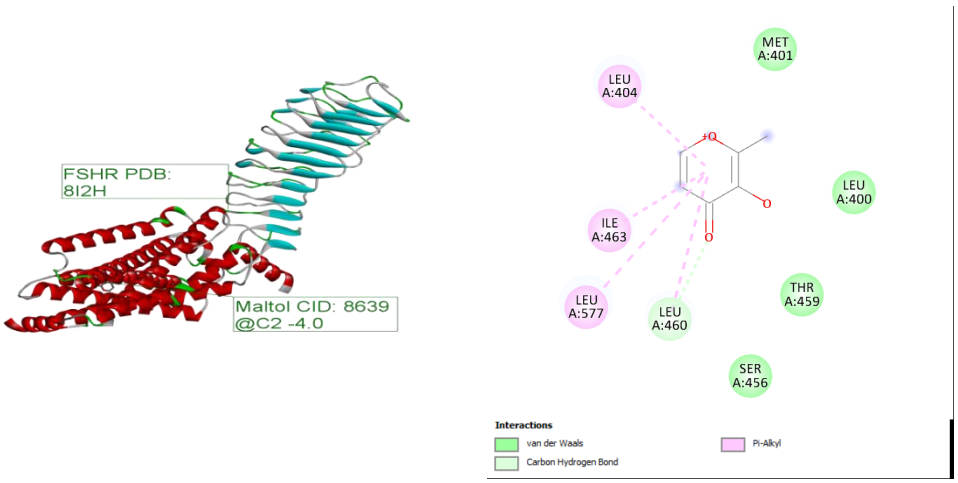



Fig 10.2: 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (Maltol CID: 8369) at positions C2 showing binding affinity and bonds at the binding site.

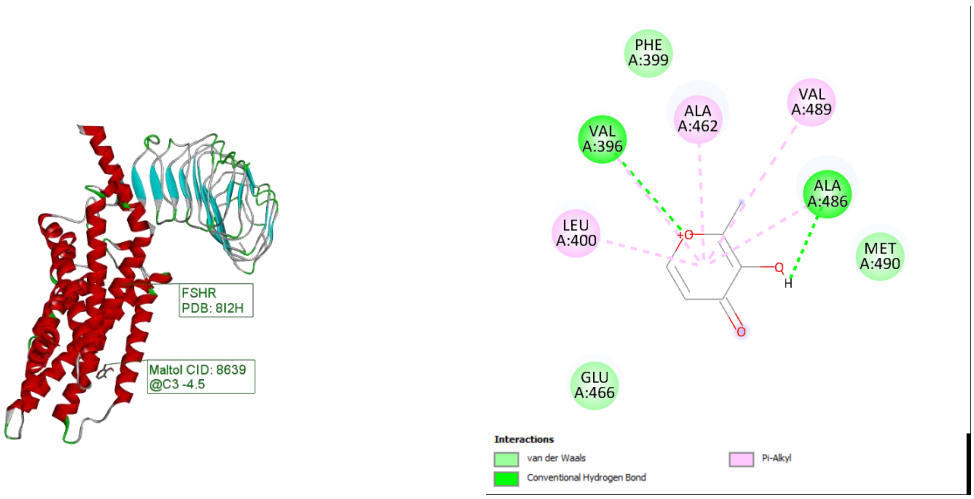



Fig 10.3: 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (Maltol CID: 8369) at positions C3 showing binding affinity and bonds at the binding site.

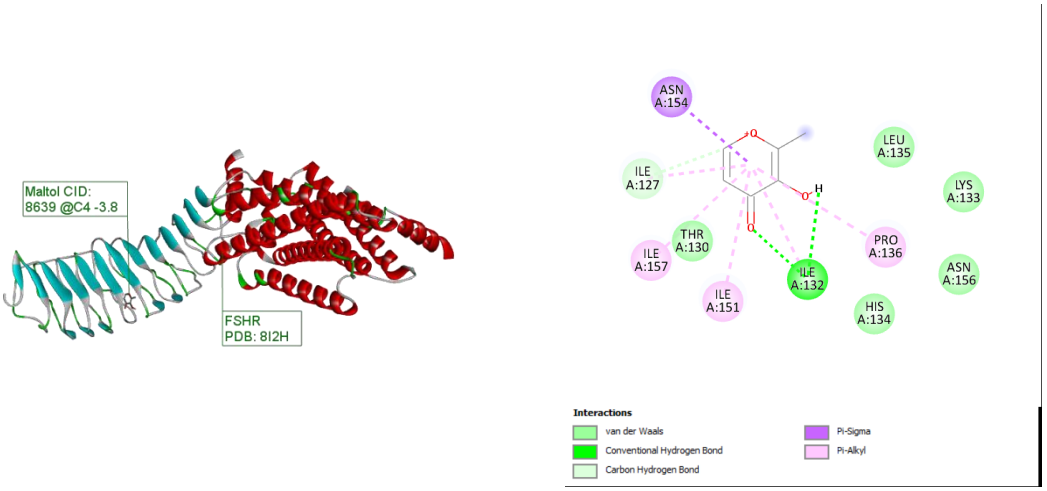



Fig 10.4: 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (Maltol CID: 8369) at positions C4 showing binding affinity and bonds at the binding site.

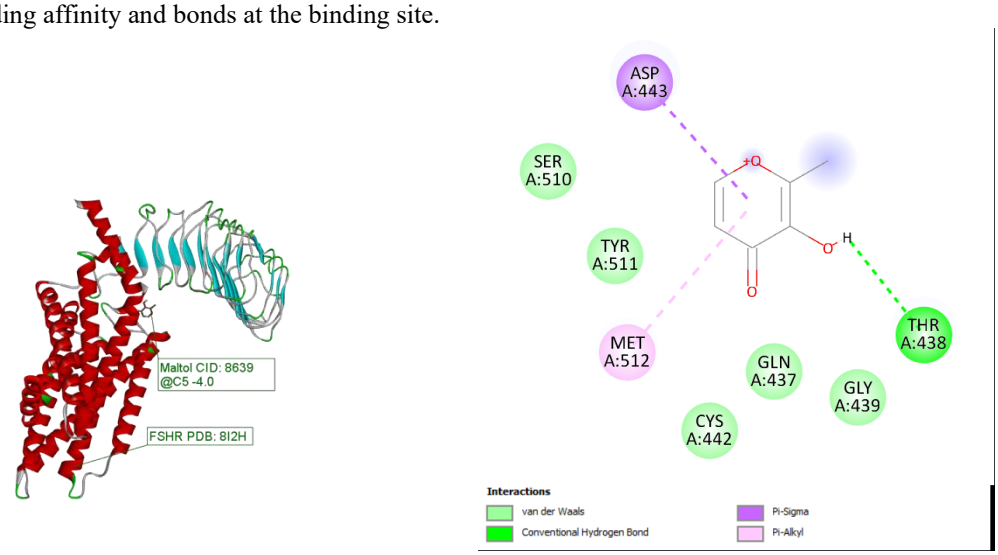



Fig 10.5: 3D and 2D IMAGE REPRESENTATIONS OF FSH receptor and ligand (Maltol CID: 8369) at positions C1 showing binding affinity and bonds at the binding site.

**Below are 3D and 2D IMAGE REPRESENTATIONS OF FSH Receptor and ligand (Urofollitropin CID: 73759969) at positions C1-C5 showing binding affinity and bonds at each binding site.**

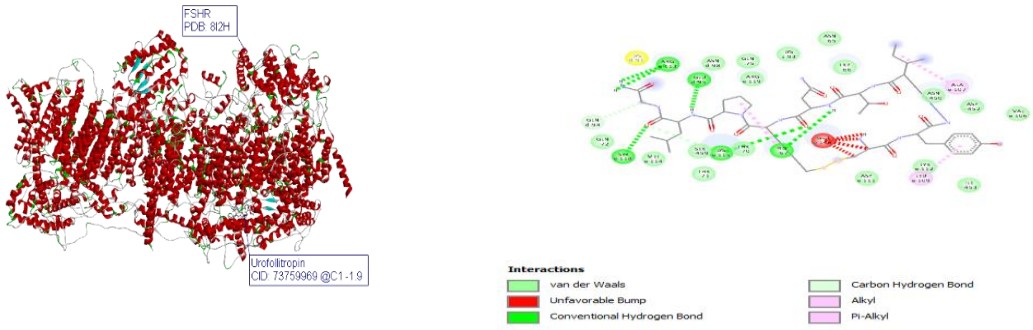



**Fig 11.1: 3D and 2D image representations of FSH Receptor and ligand (Urofollitropin CID: 73759969) at positions C1 showing binding affinity and bonds at the binding site**

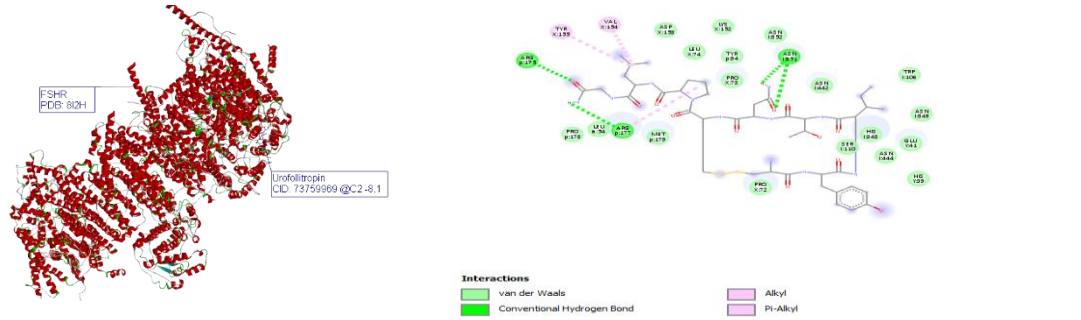



**Fig 11.2: 3D and 2D image representations of FSH Receptor and ligand (Urofollitropin CID: 73759969) at positions C2 showing binding affinity and bonds at the binding site**

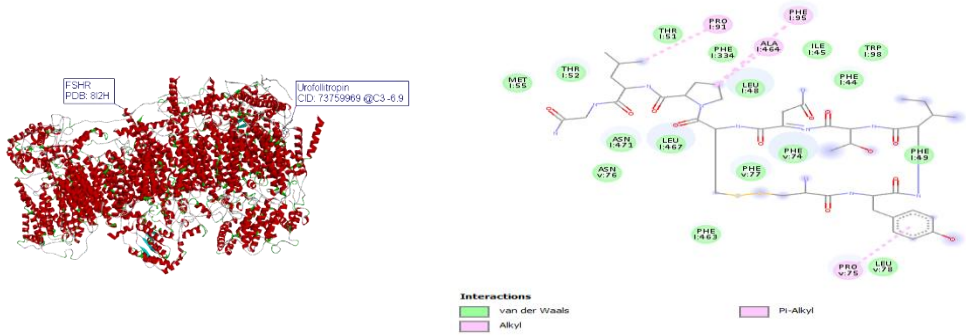



**Fig 11.3: 3D and 2D image representations of FSH Receptor and ligand (Urofollitropin CID: 73759969) at positions C3 showing binding affinity and bonds at the binding site**

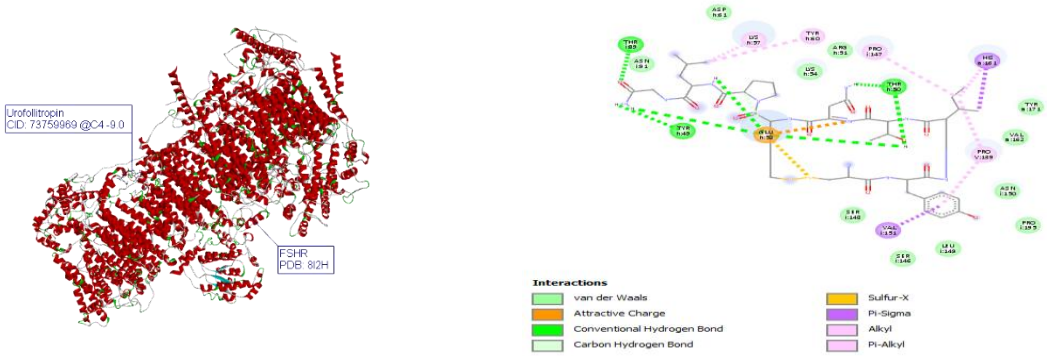



**Fig 11.4: 3D and 2D image representations of FSH Receptor and ligand (Urofollitropin CID: 73759969) at positions C4 showing binding affinity and bonds at the binding site**

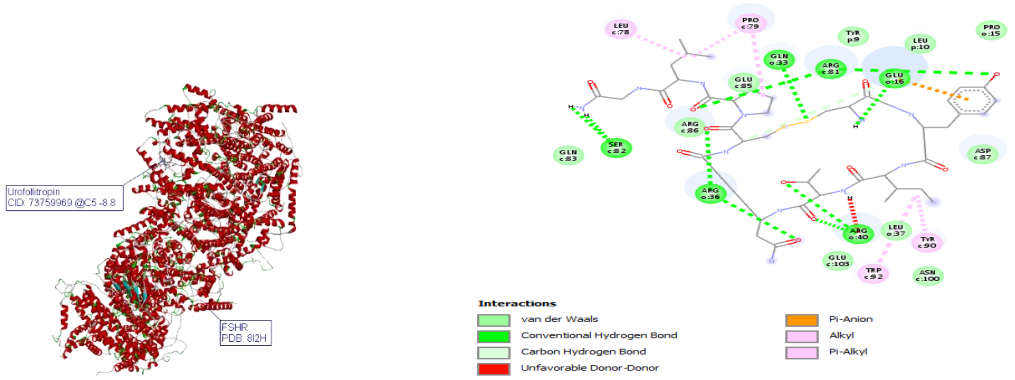



**Fig 11.5: 3D and 2D image representations of FSH Receptor and ligand (Urofollitropin CID: 73759969) at positions C5 showing binding affinity and bonds at the binding site**

The docking results of CID 7311 and CID 8369 on FSH receptor (PDB 8I2H) showed that the binding site at position C3 possessed the highest binding affinity with vina score of -5.9 for CID 7311 and -4.5 for CID 8369, the 2D representations showed presence of 1 H bond and 2 H bonds respectively.

GC-MS result on the phytoconstituents present in the stem juice of *Costus afer* stem juice showed 2-Furancaboxaldehyde, 5-(hydroxymethyl)- as the most abundant phytoconstituent with a %area of 70.357% forming one of the major constituents in the juice and ethyl, β-d-riboside with a %area of 0.149% having the least quantity. Both 2-Furancaboxaldehyde and 5-(hydroxymethyl)- have been identified as a hematoprotective agent and in inhibiting virulence factors of *C.* albican such as morphological transition as well as secreting hydrolase production [22]. The bio-active constitutnts of *Costus afer* leaves extract was also investigated which revealed the presence of seventy-seven (77) constituents with 2,4-Di-tert-butylphenolas the most abundant constituent with a %area of8.08% and 3-Eicosene, (E)- as the least abundant with peak areas of 0.15%. The 2, 4-di-tert-butylphenol, which is one of the phenolic antioxidants and is routinely used as an intermediate for the preparation of antioxidants and ultra-violet (UV) stabilizers, or in the manufacturing of pharmaceuticals and fragrances. Phenolic compounds are commonly found in plants [23] (Choi *et. al.,* 2013). The potential protective role of phenolic compounds against oxidative damage-induced diseases that can be provided by the consumption of fruits, vegetables, and herbs has drawn considerable research interest. The importance of antioxidant activity of phenolic compounds and their possible use in processed foods as a natural antioxidant, notably, phenolic compounds have been shown to exert potent antioxidant effects [24, 25] (Jang *et. al.,* 2007; Dasha *et. al.,* 2024). Thus 2,4-di-tert-butylphenol has also been reported to have other medical properties such as, anticancer [26] (Sri-Nurestri *et. al.,* 2009), antifungal [27] (Varsha *et. al.,* 2015) and antibacterial activities (Rashmi *et. al.,* 2020). The GC-MS analysis of the *Costus afer* root extract was also investigated and showed the presence of forty-two (42) bio-active constituents with 2,4-di-tert-butylphenol as the most abundant.

Molecular docking results revealed the presence of hydrogen bonds to suggests that there is a sufficient interaction between follicle stimulating hormone receptor (PDB 8I2H) and the ligand compounds, viz; 2,4-Di-tert-butylphenol and Maltol as it exist in urofollitropin which is a standard drug.

These interactions will supposedly illicit reactions on the protein receptor, which may in turn, affect the expression of FSH in the body [29] (Alfredo *et al.,* 2025). Thereby suggesting that the use of these plant extracts can regulate expression of FSH because of presence of these active compounds.

## V. Conclusion

The GC-MS results of the crude stem juice extract of *Costus afer*, aqueous leaf extract of *Costus afer* and aqueous root extract of *Costus afer* have revealed some of the bio-active components of the plant. Most of these chemical components have considerable therapeutic value. This study also investigates the therapeutic effects of *Costus afer* parts on reproduction function using the instrumentality of molecular docking which revealed their binding affinity on FSH receptor. Based on the critical roles of 2,4-Di-tert-butylphenol and Maltol as anti-inflammatory agents to bring about positive effects on reproduction in inflammation. These phytochemicals; 2,4-Di-tert-butylphenol and Maltol are potential evolving remedies for preventing and treating infertility in male and female respectively. The results show that *Costus afer* may be an important reservoir for developing affordable fertility drugs.

**Abbreviations**

GC-MS Gas Chromatography-Mass Spectrometry

**Acknowledgements**
The authors wish to acknowledge the assistance of the Department of Biochemistry, Abia State University Uturu for giving us access to their laboratory facilities in the course of the research.
Conflict of Interest
Authors have declared that no conflicts of interest exist.

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